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THE PROBLEM OF THE MECHANISM OF ACTION OF PYRIDOXINE (VITAMIN B6) IN ACUTE RADIATION INJURY

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Pyridoxine (vitamin B6) is associated with the protein and fat metabolism (1-4, 8, 10, 12, 15, 23), participates in the biosynthesis of nicotinic acid (1,3), exerts an influence on hematopoiesis (17, 22, 25) and on the normalization of the gastrointestinal tract and liver function (11), detoxifies histamine (3,6). Because of this, the application of pyridoxine as a prophylactic and therapeutic agent has a sound basis in acute radiation sickness.

The data in the literature on this question, however, are sparse and contradictory. Some authors consider pyridoxine the best therapeutic agent in radiation sickness (27, 30); others assert that this preparation is effective only under certain conditions--in avitaminosis and when it is administered only before irradiation or in the latent period (7,14, 16, 20, 21, 28); still others even note a negative influence, which is exerted by pyridoxine and expressed in a reduction in the hemoglobin content in the blood (18).

In this investigation we have tried to clarify the time in acute radiation injury that the use of pyridoxine is most expedient and to study a certain mechanisms of its action in the irradiated organism.

Experiments for the clarification of the effect of pyridoxine on the course and outcome of acute radiation injury were performed on 160 rabbits, 860 rats and 80 guinea pigs. The rabbits and rats were irradiated once with Co^{60} gamma rays in a dose of 800 r; the guinea pigs, with a dose of 450 r. The dose rate amounted to 10-15 r a minute. The pyridoxine was administered orally in aqueous solution in a dose of 0.3-three mg/kg. In addition, several variants of experiments for the study of the mechanism of action of pyridoxine in the irradiated organism were performed on 240 rats and 67 rabbits. With this aim in view the pyridoxine was used in the acute period of acute radiation sickness simultaneously with the antihistamine, dimedrol (benadryl), and the comparative effects of the repeated injections of pyridoxine (intravenously in a dose of one mg/kg) and of a small dose of histamine (10 gamma/kg) before and after irradiation on the histaminase activity in the blood plasma were investigated. The histamine and pyridoxine were injected daily: for a week to non-irradiated rabbits; for a week before the irradiation or from the seventh to the 11th day after it in those irradiated with 500 r. Blood for the purpose of obtaining plasma was taken from the non-irradiated rabbits on the 10th and 18th days after beginning the administration of the preparations; from the experimental animals, at different intervals, from the first to 20th day after irradiation.

The effect of the simultaneous application of pyridoxine with the dimedrol (5-15 gamma/kg) was studied during the acute period of the radiation sickness in rats (survival rate) and rabbits (histaminase activity in the plasma). The preparations were injected intraperitoneally from the seventh to the 11th day after the irradiation (with glucose and ascorbic acid for the purpose of eliminating side-effects of the dimedrol). The control animals received dimedrol with glucose and vitamin C or physiological solution.

The histaminase activity in the plasma of the irradiated and non-irradiated rabbits (controls, that is, those which had not been given any preparations, and the experimental rabbits, that is, those which had been given histamine, pyridoxine or pyridoxine with dimedrol) were investigated for the effect of the plasma being tested on the histamine-produced reduction in arterial pressure in a cat, utilizing the Kluga (9) method.

The results of the survival rates of the animals and the data on the histaminase activity were treated mathematically by the methods of variation statistics. The reliability of the data of the histaminase activity was appraised by the error in the difference between the absolute values and the criterion "t"; the reliability of the differences in survival rate were evaluated by the error in the difference between the relative values and the criterion, χ^2.

The use of pyridoxine at different times before and after the irradiation exerted different effects.

In three series of experiments the rabbits irradiated with 800 r were injected with 0.3 mg/kg of pyridoxine for a week before the irradiation (series I), for two weeks after the irradiation (series II), for a week before and two weeks after the irradiation (series III). The administration of the preparation after the irradiation alone led to a reduction in the survival rate of the rabbits by 15 percent, and the administration of it before and after the irradiation, to an increase in the survival rate by 20-35 percent.

Thus, of the 20 experimental rabbits irradiated with 800 r in series II only five (25 percent) survived, while of the 20 control, non-irradiated rabbits, eight survived (40 percent). Of the 40 experimental rabbits of series I 38 (95 percent) survived, while of the 40 control animals, 30 (75 percent) survived; in series III 14 of the 20 (70 percent) experimental animals survived and seven out of 20 (35 percent) of the control rabbits. The error in the difference of the data in series II was equal to 1.0;

in series I, 2.86; and in series III, 2.33.

The rabbits which had been given pyridoxine before the irradiation did not die at all from the shock-like phenomena shortly after irradiation, whereas in the control group 15-20 percent of the animals died after irradiation. In the surviving animals which were given vitamins before and after irradiation there was less of a weight drop by comparison with the controls, and the various blood indices were better (Figs 1 and 2). In the rabbits given vitamins only after irradiation, the weight decreased more considerably during the experimental than in the controls, and a more pronounced depression of erythrocytopoiesis was observed (Figs. 3 and 4).

Experiments on rats, as on rabbits, also showed the importance of the time at which the pyridoxine was used (see Table).

As seen from the Table, the repeated injection of pyridoxine into rats before the irradiation (series II, group 1) as well as the combination of the injection of pyridoxine before irradiation and at various times after it (series VI and VII) exert a definite protective effect, improving the course of radiation sickness and producing an increase in the survival rate by 12-36 percent. The single administration of large doses of pyridoxine a week before the irradiation and directly before it proved to be ineffective (series I).

We observed
/The greatest protective effect after the repeated prophylactic use of pyridoxine in rabbits and rats in a series of experiments with a low survival rate in the control group which was carried out in the winter-spring period (rabbits, series III; rats, series VII). This was apparently associated with the elimination of a moderative vitamin deficiency which occurs in the animals in the spring, and which is not at all manifested clinically without irradiation, but leads to a considerable reduction in the survival rate of the irradiated animals.

The use of pyridoxine in experiments on rats only after irradiation exerts different effects depending on the time of its application. The injection of it in the latent period of the disease from the first to the fifth day (series IV) and during the recovery period, from the 14th to 21st day (series II, group 2) somewhat improves the course of the sickness and increases the survival rate by four to eight percent. The use of pyridoxine throughout the entire acute period leads to an impoverishment of the results with an increase in the mortality rate by 16 percent (series III). Mathematical treatment of the total data on the reduction of the mx mx survival rate of rabbits of series II and rats of series III which were given pyridoxine during the acute period of radiation sickness shows the statistical reliability of this reduction: the error in the difference between the experimental and control animals is 2.0.

A harmful effect was also observed when the pyridoxine was administered both before and after irradiation if the vitaminization was carried out for a long time after the irradiation--throughout the entire latent and acute periods (see Table series V).

When pyridoxine was . administered to guinea pigs before and after irradiation the data concerning the greater efficacy of the prophylactic effect of this preparation were mx :xx confirmed; the survival rate of the guinea pigs vitaminized for a week before the irradiation was increased by 35 percent. The administration of pyridoxine after irradiation as well did not add anything the result obtained.

Four groups of 20 guinea pigs each were irradiated with 450 r and were given 0.3 mg/kg of pyridoxine each. Of the group of animals vitaminized for a week before the irradiation 13 survived (65 percent); of those vitaminized for a week before and from the first to the fifth day after irradiation, 12 (60 percent); and of the x guinea pigs vitaminized for a week before and from the eighth to the 21st day after irradiation, 13 (65 percent). Of the control guinea pigs which were g not given pyridoxine, six survived (30 percent). The errors of the data in the three experimental groups

by comparison with the control were 2.14, 2.0 and 2.14, respectively.

Simultaneous use of pyridoxine and dimedrol during the acute period of radiation sickness increased the survival rate of the rats to 71.2 percent (of the 80 rats 57 survived), whereas of the 80 control animals which had been given physiological solution intraperitoneally only 43 (53.7 percent) survived. This difference is statistically reliable: the error in the difference is 2.3. Of the group of 80 rats which were injected with dimedrol without pyridoxine 53 survived (66.2 percent).

The histaminase activity was increased both in the plasma of rabbits which had been given pyridoxine repeatedly and in the plasma of rabbits which had been given small doses of histamine.

The plasma of both groups of experimental rabbits, mixed and kept in a water bath with histamine, decreased the fall in arterial pressure of the ox brought about by pure histamine in the same dose, by an average of 13.4 and 12.3 mm respectively; the plasma of the control rabbits decreased it by only 7.5 mm. These differences were confirmed statistically. The error in the difference for the vitaminized and control rabbits was 3.14; for the animals given histamine and for the controls, 2.45.

In the irradiated rabbits which had been given pyridoxine or histamine for a week before the irradiation the content of histaminase in the blood plasma at different intervals after the irradiation was also higher than in the controls. While the plasma of rabbits given pyridoxin reduces the drop in arterial pressure caused by histamine by an average of 16.1 mm and the plasma of rabbits preliminarily treated with histamine reduces it by 11.2 mm, the plasma of control animals reduces this drop by only 8.5 mm. The error of the difference for the vitaminized and control rabbits in these experiments was 2.87.

The administration of pyridoxine during the acute period of radiation sickness as well as administration of histamine led to a reduction in the activity of histaminase in the plasma.

While
/the plasma of control rabbits mixed with histamine reduced the drop in arterial pressure in the cat caused by the same doses of pure histamine by an average of 6.25 mm, the plasma of rabbits given histamine during the acute period of radiation sickness reduced it by 2.7 mm, and the plasma of vitaminized rabbits, by only 0.75 mm. The error in the difference between /the group of rabbits given histamine and the controls was 2.5; for the vitaminized and controls, 3.76.

The simultaneous administration of pyridoxine and dimedrol during the acute period of radiation sickness, however, eliminated the reduction in histaminase activity in the plasma of rabbits which had occurred as the result of the use of pyridoxine alone. The histaminase activity in animals given pyridoxine with the dimedrol became almost the same as the histaminase activity in the control animals: the plasma of the experimental rabbits reduced the blood pressure drop in the cat produced by histamine by an average of 5.6 mm; the plasma of the between controls, by 6.25 mm. The error of the difference /rabbits given pyridoxine with dimedrol and rabbits given only pyridoxine was 2.90.

Therefore, the repeated administration of pyridoxine before irradiation in various species of animals exerts a protective effect: it protects against shock, improves the general condition, increases the survival rate. The single injection either of a large dose of pyridoxine /a week before irradiation or directly before it does not exert any protective effect. With the administration of pyridoxine after irradiation success is variable. The deterioration in the outcome of radiation sickness in animals given vitamins during the acute period attracts attention.

As is well known, phosphopyridoxal--a pyridoxine derivative--is associated simultaneously with the processes of histamine formation and destruction in the animal organism. By means of decarboxylation of histidine it contributes to the formation of histamine, while since it is a histaminase coenzyme it participates

in its destruction (2,3, 19, 29, 31).

In the radiobiological literature there are works in existence the authors of which succeeded in finding an increased histamine content in the body after irradiation (5, 13, 24, 26). Unsuccessful attempts to find histamine in the blood do not eliminate the possibilities of increasing its content, because even at the height of the artificial histamine shock histamine cannot always be found in the blood (19). Apparently, it is readily detected only after a considerable reduction in the histaminase activity in the body. Its activity varies depending on the total dose and the duration of the irradiation, the period of radiation sickness and the original quantity of the enzyme. Whatever the role of histamine in the pathogenesis of radiation injury, however, the administration of histamine or substances possessing a histamine-forming capacity cannot help but have a harmful influence on the outcome of the disease in the most severe, acute period of radiation sickness. In our experiments with pyridoxine an impoverishment in the outcome of the radiation injury after the administration of the vitamin in the acute period was actually observed. Here, the activity of histaminase in the plasma of vitaminized rabbits was reduced, and the administration of pyridoxine with the antihistamine preparation dimedrol increased this activity and lengthened the life of the animal.

Apparently, the reduction in histaminase activity produced by the pyridoxine was caused specifically by the property of this vitamin of forming histamine, which to some degree inactivates the histaminase and can contribute to the deterioration in the outcomes of the disease. The simultaneous administration of dimedrol reduces this effect.

The protective effect of pyridoxine when it is used prophylactically is apparently also associated, to a considerable degree, with its histamine-forming function, which is confirmed by the increase in histaminase activity in the blood plasma after the

repeated injection of either pyridoxine or small doses of histamine to the animals. However, this protective effect cannot be attributed merely to an increase in the activity of the enzyme which destroys histamine. Thus, when blood is taken repeatedly from the he for the purpose of obtaining plasma in irradiated rabbits we observed that in the animals given histamine a more marked reduction in histaminase activity is found than in the vitaminized rabbits. In addition, the repeated paranteral administration of pyridoxine to rabbits and rats protected them from histamine shock and histamine lymphocytopenia more effectively than the similar administration of histamine. Evidently, the protective effect of pyridoxine was produced not only by its histamine-forming but also by its other functions.

Based on the data which we obtained the use of pyridoxine in acute radiation injuries may be recommended as a prophylactic measure as well as in the capacity of a therapeutic measure in the latent period and during the recovery period of radiation sickness; the administration of pyridoxine in the acute period without combining it with other therapeutic preparations is undesirable.

Conclusions

1. The use of pyridoxine before irradiation in various species of animals (rabbits, rats, guinea pigs) exerts a pronounced protective effect.

2. The administration of pyridoxine during the acute period of radiation injury causes a deterioration in the course and outcome of the sickness.

3. The administration of pyridoxine before irradiation and the administration of small doses of histamine increases the histaminase content in the blood plasma, and the administration of these preparations in the acute period of radiation sickness reduces the activity of the enzyme.

4. The use of/the antihistamine preparation dimedrol during the acute period of radiation sickness simultaneously with pyridoxine eliminates the harmful effect of the

pyridoxine on the course and outcome of the sickness and prevents a reduction in the blood histaminase level.

5. The protective effect of pyridoxine when used prophylactically and the harmful effect when the vitamin is administered during the acute period of radiation sickness are to a large degree associated with the histamine-forming function of pyridoxine and with the content of histaminase in the body which destroys histamine.

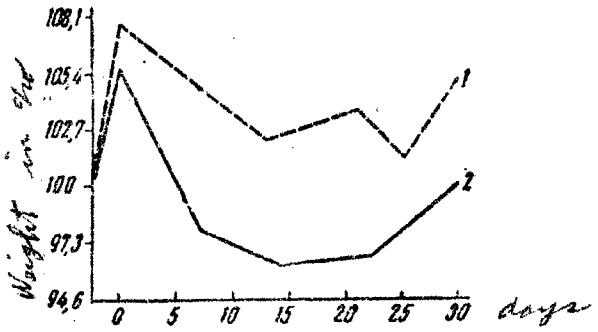

Fig. 1. Change in weight of rabbits given pyridoxin before irradiation. 1) vitaminized; 2) controls.

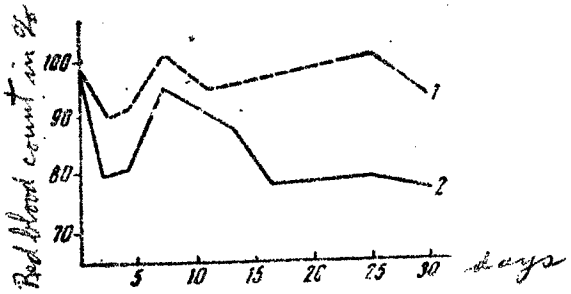

Fig. 2. Change in red blood count of rabbits given pyridoxine before irradiation. Key same as for Fig. 1.

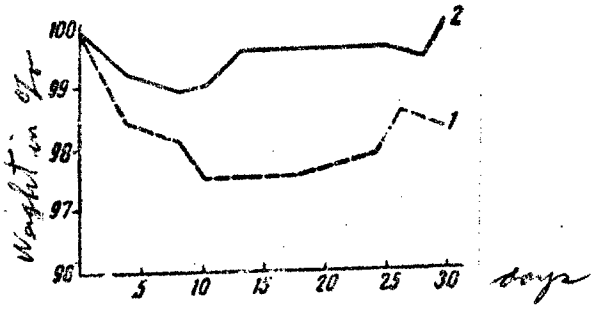

Fig. 3. Change in weight of rabbits given pyridoxine after irradiation. Key is the same as in Fig. 1.

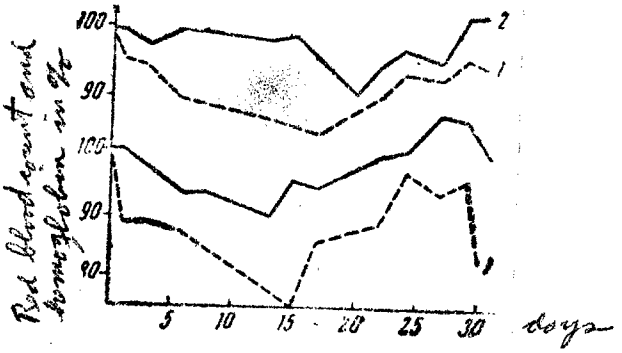

Fig. 4. Change in red blood count and hemoglobin in rabbits given pyridoxine after irradiation. Key same as for Fig. 1.

Change in the Survival Rate of Rats Irradiated With 800 r With the Use of Pyridoxine

No of series	No of group	No of animals	Доза пиридоксина в мг/кг (1)	Time at which used	No of surviving animals		Ошибка разности опыта и контроля (2)
					abs	%	
I	1	60	20,0	Once a week before irradiation	27	45,0	
	2	60	20,0	Once directly before irradiation	22	36,7	0,43
	3	30	—	Control	13	43,3	
II	1	50	3,0	В течение недели до облучения (3)	28	56,0	
	2	50	3,0	(4) 14-го до 21-го дня после облучения	19	38,0	
	3	50	—	Контроль (5)	15	30,0	2,71 0,9
III	1	50	3,0	From the 6th to 21st day after irradiation	18	36,0	
	2	50	—	Control	26	52,0	1,8
IV	1	50	3,0	From the 1st to 5th day after irradiation	10	20,0	
	2	50	—	Control	8	16,0	0,66
V	1	30	1,0	For a week before and three weeks after irradiation.	13	43,3	
	2	30	—	Control	15	50,0	1,3
VI	1	50	3,0	For a week before and 2 weeks after irradiation	22	44,0	1,33
	2	50	0,3	For a week before and 2 weeks after irradiation	22	44,0	
	3	50	—	Control	16	32,0	1,33
VII	1	50	1,0	(6) В течение недели до и с 1-го до 5-го дня после облучения	27	54,0	4,11
	2	50	1,0	(7) В течение недели до и с 8-го до 21-го дня после облучения	18	36,0	
	3	50	—	(8) Контроль	9	18,0	2,07

1) Dose of pyridoxine in mg/kg; 2) Error of difference of experimental and control groups; 3) For a week before irradiation; 4) From the 14th to the 21st day after irradiation; 5) Control; 6) For a week before and from the 1st to 5th day after irradiation; 7) For a week before and from the 8th to 21st day after irradiation.

NOTE: In series of experiments in which there were 2 groups of experimental rats and a single control, the error in the difference for the vitaminized animals was computed with respect to the same controls.

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